MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2017

July–September 2017—Volume 132, pp. 635–642

https://doi.org/10.5248/132.635

Tuber magnatum in Thailand, a first report from Asia

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Abstract—Specimens collected in Thailand have been identified as *Tuber magnatum*, based on morphology and phylogenetic analysis of the LSU and ITS sequences. A description and illustration are provided. This is the first record of this fungus from Asia.

Key words—ascomycete, hypogeous fungi, taxonomy, white truffle

Introduction

Truffles (*Tuber* spp., *Tuberaceae*, *Pezizales*) are well known as the most expensive edible fungi in the world (Hall et al. 2003, 2007; Zambonelli et al. 2016). The Italian white truffle (*T. magnatum*), Périgord black truffle (*T. melanosporum* Vittad.), and summer truffle (*T. aestivum* Vittad.) are highly prized foods in Europe (Hall et al. 2007; Stobbe et al. 2013). In North America, Oregon white truffles (*T. oregonense* Trappe et al. and *T. gibbosum* Harkn.) are commercially harvested (Bonito et al. 2011). An Asian black truffle, *T. indicum* Cooke & Massee (the first Asian truffle reported from India; Cooke 1892), is one of the renowned commercial species in China (Mortimer et al. 2012). Since the 1980s, more than 20 new *Tuber* species have been discovered in China, Japan, and Taiwan (Liu & Liu 1994; Tao 1988; García-Montero et al. 2010; Fan et al. 2011a,b, 2012a,b, 2014, 2015; Fan & Cao 2012; Deng et al. 2013; Fan & Yue 2013; Kinoshita et al. 2016), and two new truffles, *T. thailandicum* N. Suwannarach et al. and *T. lannaense* N. Suwannarach & Lumyong have been described from Thailand (Suwannarach et al. 2015, 2016).

During an investigation of hypogenous fungi in northern Thailand, we found specimens that corresponded to the description of *T. magnatum,* a species unknown in Asia and previously reported from Croatia, Hungary, Italy, Serbia, Slovenia, and Switzerland (Hall et al. 1998, 2007; Mello et al. 2005; Rubini et al. 2005; Figliuolo et al. 2013). We describe and illustrate the morphological characters of the Thai material and provide results from molecular phylogenetic analyses.

Materials & methods

Morphology studies

Ascomata were collected under *Carpinus* sp. (*Betulaceae*) in a mixed evergreen hill forest in northern Thailand. Macromorphological descriptions were made within 24 h after collection. Color names and codes follow Kornerup & Wanscher (1978). The specimens were dried at 40–45 °C; sections of dried material were mounted in 95% ethanol and rehydrated in distilled water, 3% KOH, or Melzer's reagent for microscopical examination; at least 50 measurements were made of each structure. *Q* is the length to width ratio and **Q** is the average *Q* of all specimens ± standard deviation. The collections are deposited at the Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Molecular studies

Genomic DNA of three specimens was extracted from fresh tissue using the Genomic DNA Extraction Mini Kit (Favogen, Taiwan). The large subunit (LSU) of nuclear ribosomal DNA was amplified by polymerase chain reaction (PCR) with LROR and LR5 primers under the following thermal conditions: 95 °C for 2 min; 30 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. The internal transcribed spacer (ITS) region of ribosomal RNA gene was amplified with ITS4 and ITS5 primers under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light and purified using NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Germany) following the manufacturer's protocol. The purified PCR products were directly sequenced. Sanger sequencing was performed by 1ST Base Company (Kembangan, Malaysia). Sequences were used to query GenBank database via BLAST (http://blast.ddbj.nig.ac.jp/top-e.html).

The sequences for phylogenetic analysis were obtained in this study and from GenBank database. The multiple sequence alignment was carried out using MUSCLE (Edgar 2004). A maximum likelihood (ML) phylogenetic tree was constructed using RAxML v7.0.3 (Stamatakis 2006), applying the rapid bootstrapping algorithm for 1000 replications using the GTRGAMMA model. *Choiromyces meandriformis* Vittad. and *C. alveolatus* (Harkn.) Trappe were used as the outgroup. The ML trees were viewed with TreeView32 (Page 2001). Bayesian phylogenetic analysis was carried out using

the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method in MrBayes version 3.2 (Ronquist et al. 2012).

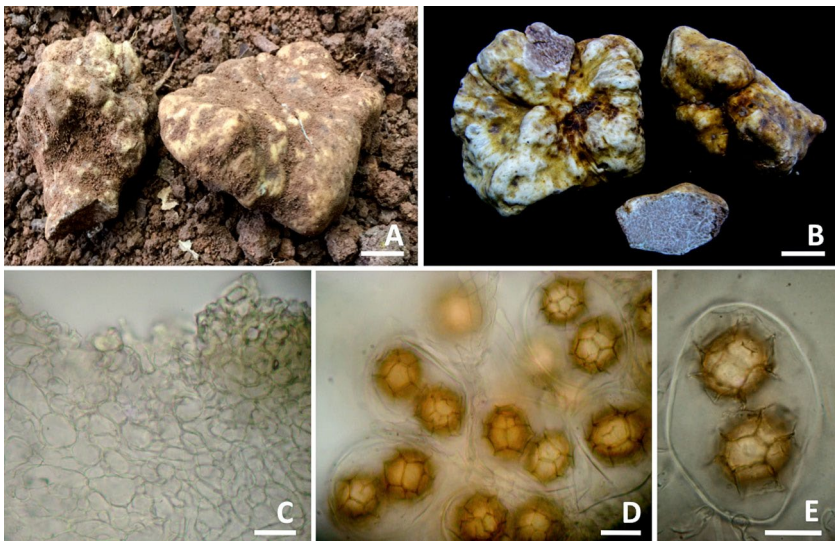

Fig. 1. *Tuber magnatum* (SDBR-CMU-MTUF010). A, B: Ascomata; C: Peridium with pseudoparenchymatous tissues; D, E: Asci and ascospores as observed under a compound microscope. Scale bars: A, B = 10 mm; C = 10 µm; D, E = 25 µm.

Results

Taxonomic description

Tuber magnatum Picco, Meleth. Bot.: 79 (1788). Fig. 1

Ascomata hypogeous, irregular in form, lobed, gibbous, sometimes flattened, 23–65 mm diam., white (2A1) to pale yellow (4A3) when fresh, gradually becoming pale yellow (4A3) to brown (6D8) when dry, surface smooth to verrucose, glabrous. Odor strongly aromatic when mature. Peridium 250–500 µm thick, composed of two layers. Outer layer 150–300 µm thick, pseudoparenchymatous, composed of isodiametric cells, pale yellow or hyaline, 5–25 µm diam., cell walls 0.5–1.5 µm thick; hyphae scattered, hyaline, thin-walled, 2–5 µm diam.. Inner layer 100–200 µm thick, hyphae intricately interwoven, hyaline, 2–5 µm diam., thin-walled. Gleba solid, white when young, becoming pale yellow, reddish brown, often with flesh-red spots, marbled with numerous meandering white veins. Asci globose to subglobose or ellipsoid, hyaline, with thin or slightly thickened walls, 55–125 × 40–115

μm, sessile with short stalk 2–5 μm long, 1–4-spored (mostly 2-spored). Ascospores subglobose to broadly ellipsoid, hyaline when young, becoming light yellow to yellowish brown at maturity; excluding their alveolate-recticulate ornamentation 30–40 × 27.5–32.5 μm, *Q* = 1.08–1.33 in 1-spored asci, 26.5–30 × 22.5–25 μm, *Q* = 1.05–1.20 in 2-spored asci, 22.5–25 × 21–23.75 μm, *Q* = 1.00–1.25 in 3-spored asci, 17–23.75 × 17–22.5 μm, *Q* = 1.00–1.21 in 4-spored asci, **Q** = 1.13 ± 0.09; spines 1.5–7.5 μm high, constituting mostly of hexagonal meshes 2–3 across the spore width, 12.5–17.5 × 5–12.5 μm,.

Specimens examined—**THAILAND**, **Chiang Mai Province**, Muang District, Doi Suthep-Pui National Park, Doi Pui, 18°49′52″N 98°54′12″E, elevation 1580 m, under *Carpinus* sp. (*Betulaceae*) in mixed evergreen hill forest, sandy loam, 25 July 2016,

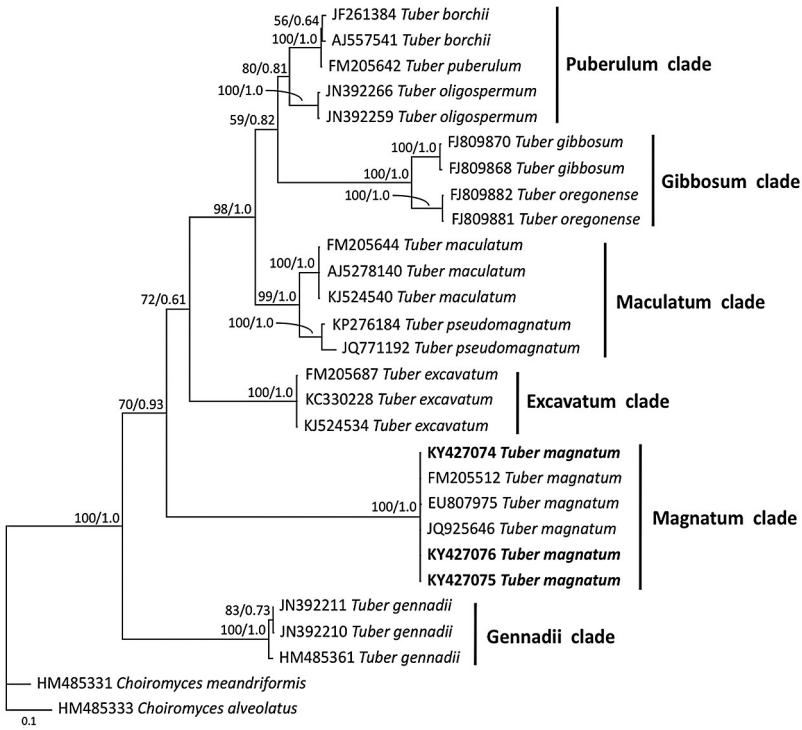

Fig. 2. Phylogram derived from maximum likelihood analysis of the LSU region of nuclear rDNA from 30 sequences. *Choiromyces alveolatus* and *C. meandriformis* were used as the outgroup. The numbers above branches represent maximum likelihood bootstrap percentages (left) and Bayesian posterior probabilities (right). Only bootstrap values ≥50 % are shown. The sequences obtained from this study are in bold.

Suwannarach N. (SDBR-CMU-MTUF010; GenBank KY427071, KY427074); 3 August 2016, Suwannarach N. & Kumla J. (SDBR-CMU-MTUF011; GenBank KY427072, KY427073); 25 August 2016, Suwannarach N. & Kumla J. (SDBR-CMU-MTUF012; GenBank KY427075, KY427076).

Molecular analysis

Bonito et al. (2011) identified six main clades (Excavatum, Gennadii, Gibbosum, Maculatum, Magnatum, and Puberulum) within *Tuber*. Our ITS- and LSU-based phylograms (Figs 2, 3) place the Thai specimens within the monophyletic Magnatum clade with high bootstrap (100%) support and Bayesian posterior probabilities (1.0).

Discussion

Tuber magnatum, the world's most expensive truffle, has previously been reported only from Europe (Hall et al. 1998, 2007; Mello et al. 2005; Rubini

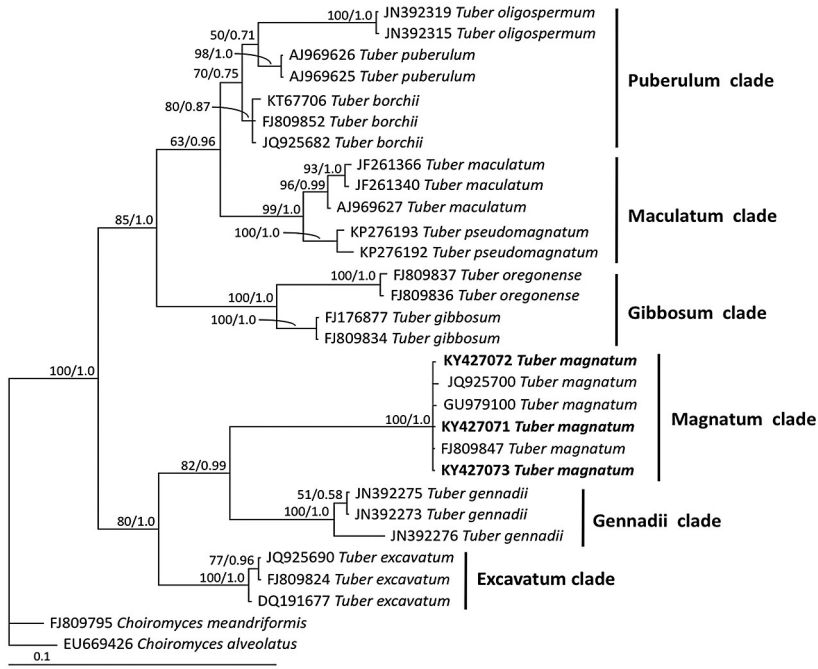

Fig. 3. Phylogram derived from maximum likelihood analysis of the ITS region of nuclear rDNA from 28 sequences. *Choiromyces alveolatus* and *C. meandriformis* were used as the outgroup. The numbers above branches represent maximum likelihood bootstrap percentages (left) and Bayesian posterior probabilities (right). Only bootstrap values ≥50 % are shown. The sequences obtained from this study are in bold.

et al. 2005; Figliuolo et al. 2013). In this study, all three specimens collected in northern Thailand were initially identified as *T. magnatum* based on descriptions by Picco (1788) and Phillips (2006). This species is distinguished from other Asian whitish truffles by its large ascoma size (Hall et al. 1998; García-Montero et al. 2010; Suwannarach et al. 2015, 2016; Zambonelli et al. 2016). *Tuber magnatum* resembles *T. borchii* Vittad., *T. gennadii* (Chatin) Pat., *T. gibbosum*, *T. maculatum* Vittad., *T. oligospermum* (Tul. & C. Tul.) Trappe, and *T. oregonense*. However, detailed morphological analyses reveal these are different species. The peridium in *T. gennadii*, *T. maculatum*, *T. oligospermum*, and *T. oregonense* is prosenchymatous, while in *T. magnatum* it is clearly pseudoparenchymatous (Mello et al. 2000, Bonito et al. 2011, Alvarado et al. 2012). The 1–6 ascospores per ascus in of *T. gibbosum* separate it from *T. magnatum* (Bonito et al. 2011). The ascospores of *T. magnatum* are smaller than those of *T. borchii* (Mello et al. 2000). *Tuber magnatum* is further distinguished from other *Tuber* species by molecular phylogenetic analyses and the unique aroma of its ascomata (Hall et al. 1998, Mello et al. 2005, Bonito et al. 2011, Zambonelli et al. 2016).

Our sequence analyses also confirmed the three Thai specimens as *T. magnatum*. The combination of morphological and molecular characters helps to establish *T. magnatum* as a new record in Thailand. This finding is important to stimulate the investigations of truffles in Thailand and may contribute to our understanding of the distribution of truffles in Asia.

Acknowledgements

This work was supported by grants from Chiang Mai University and National Science and Technology Development Agency (FDA-CO-2560-4850-TH). The collection of samples was permitted by the Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand under the document number 0907.4/4769. We are grateful Dr. Eric H.C. McKenzie and Dr. Steven L. Stephenson for their helpful comments and careful review of this article.

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